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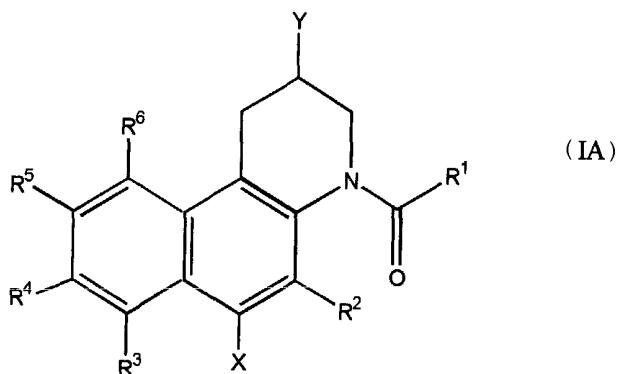
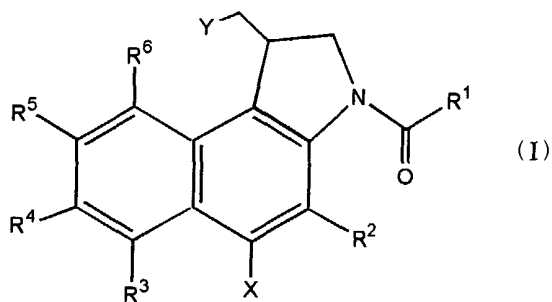
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(54) Title: BENZ-INDOLE AND BENZO-QUINOLINE DERIVATIVES AS PRODRUGS FOR TUMOR TREATMENT



(57) Abstract: Compounds of the general formula (I) or (IA) in which X is H, Y is a leaving group, R¹ preferably being an aromatic DNA binding subunit are prodrug analogues of duocarmycin. The compounds are expected to be hydroxylated at the carbon atom to which X is joined, by cytochrome P450, in particular by CYP1B1, expressed at high levels in tumours. The prodrug is expected to be activated preferentially in tumour cells, where it will act as a DNA alkylating agent preventing cell division.



For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BENZ-INDOLE AND BENZO-QUINOLINE DERIVATIVES AS PRODRUGS FOR TUMOR TREATMENT

The present invention concerns aromatic oxidation/hydroxylation activated prodrugs, particularly anti-tumour prodrugs and those which are specifically activated by the oxidation/hydroxylation activities of the cytochrome P450 family of enzymes.

Many conventional cytotoxic drugs are known that can be used for therapeutic purposes. However, they typically suffer from the problem that they are generally cytotoxic and therefore may affect cells other than those that are required to be destroyed. This can be alleviated to some extent by the use of targeted drug delivery systems, for example direct injection to a site of tumourous tissue or, e.g. binding the cytotoxic agent to an antibody that specifically recognises an antigen displayed only on the cancer cell surface. Alternatively, electromagnetic radiation may be used to cause chemical alteration in an agent at a desired site such that it becomes cytotoxic. However, all of these techniques have, to a greater or lesser extent, certain limitations and disadvantages.

The compound (+)-CC-1065 and the duocarmycins are naturally occurring representatives of a class of DNA alkylating agents. The naturally occurring compounds consist of a DNA alkylating unit based upon a pyrrolo[3,2-e]indole core, with one or two sub units, conferring DNA binding capabilities. CC-1065 and duocarmycin A comprise a spirocyclic cyclopropane group responsible for the DNA alkylation properties. Duocarmycin B₂, C₂ and D₂ are believed to be precursors for cyclopropane actives, and comprise a substituted (by a leaving group) methyl group at the eight position on the dihydro pyrrole ring. CC-1065 has been synthesised by various routes, summarised by Boger *et al* in Chem. Rev. 1997, 97, 787-828.

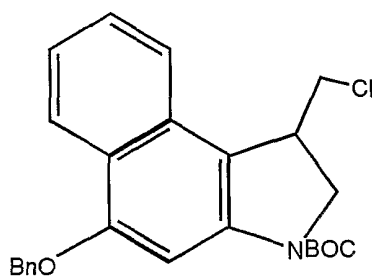
In US-A-4413132 the first synthesis of the left hand sub-unit of CC-1065 was described. The synthesis is based on a Winstein Ar-3' alkylation in which the cyclopropane ring is introduced. In a previous step, the A ring (of the indole core) is introduced by reaction of an aniline with an α -thiomethylester using chemistry based on Gassman's Oxindole Synthesis. The aniline has a protected phenolic hydroxyl group ortho to the NH₂ group,

which, in the final product, is believed to be crucial for DNA alkylation. CC-1065 has broad antitumour activity but is too toxic against normal cells to be clinically useful.

Boger *et al* (1997) *op.cit.* also describe various deep-seated structural modifications of the DNA-alkylation subunit, in which the pyrrolo 'A' ring is replaced by other aromatic ring structures. In one class of analogues the replacement ring is a benzene ring.

Attempts have been made to target the delivery of CC-1065 and analogues by conjugating the drug via the DNA binding subunit to polymers, or specific binding agents such as antibodies or biotin described in US 5,843,937. Boger *et al* in Synthesis 1999 SI, 1505-1509 described prodrugs of 1,2,9,9a-tetrahydrocyclopropa(c)benz[e]indol-4-one, in which the cyclopropane ring-opened version of the compounds were derivatised by reaction of the phenolic group to form esters and carbamates.

In Tet. Letts. (1998) 39, 2227-2230 Boger *et al.* describe (the synthesis of a range of precursors for the alkylation subunits of duocarmycin and CC-1065 analogues having a deep-seated structural modifications of the alkylation subunit. One of the compounds synthesised is a benzodihydroindole derivative:



In J. Org. Chem. (2000), 65/13, 4101-4111 the corresponding ring closed indoline compound (CBI derivative) derived from that benzodihydroindole derivative was coupled to the DNA-binding subunit of CC-1065 and shown to have DNA alkylation activity. Analogues of the alkylation subunit precursor in which the benz moiety is substituted with methoxy or cyano were also synthesised. Similar compounds are described in WO-A-9745411 and WO-A-9732850. In '411 the benz moiety is

substituted by cyano at the 7-position or the cyclopropane group may be difluoro substituted. In '850 the 7-methoxy CBI compounds are described.

In WO-A-9811101 the phenolic hydroxyl group in the B ring of CBI-type compounds is replaced by amino, nitro or thiol-based groups.

5 In J.Am.Chem.Soc. (1991), 113, 3980-3983 Boger *et al* describe a study to identify features of CC-1065 analogues contributing to the selectivity of the DNA-alkylation. The compounds tested *in vitro* had alkylating subunits based on 2,3-dihydroindole and included the 6-
10 deshydroxy analogues. These were shown to have some DNA alkylating properties though at concentrations 10^4 times higher than that of the 6-hydroxy compounds.

The present invention relates to precursors of analogues CC-1065, which do not have the hydroxyl group in the B ring of the alkylating sub unit, and which are hence substantially inactive as DNA alkylating agents
15 themselves.

It has been reported (Murray, G.I. *et al.*, 15 July 1997, Cancer Research, 57m 3026-3031 and WO-A-9712246) that the enzyme CYP1B1, a member of the cytochrome P450 (CYP) family of xenobiotic metabolising enzymes, is expressed at a high frequency in a range of human cancers,
20 including cancers of the breast, colon, lung, oesophagus, skin, lymph node, brain and testes, and that it is not detectable in normal tissues. This led to the conclusion that the expression of cytochrome P450 isoforms in tumour cells provides a molecular target for the development of new antitumour drugs that could be selectively activated by the CYP enzymes in tumour
25 cells, although no drug examples were given. A number of other CYP isoforms have been shown to be expressed in various tumours. Many of the CYP's expressed in tumours are mentioned in Patterson, LH *et al*, (1999) Anticancer Drug Des. 14(6), 473-486.

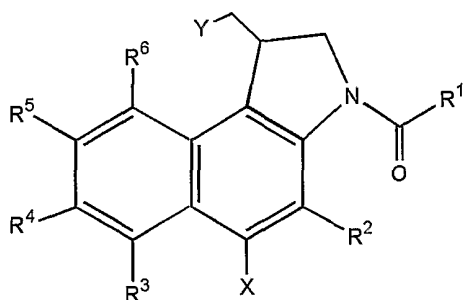
In WO-A-99/40056 prodrugs of styrene- and chalcone-derivatives are
30 described. The respective hydroxylated forms of the prodrugs, formed *in situ*, are potent tyrosine kinase (TK) inhibitors. Inhibition of TK activity contributes to tumour inhibition and cell destruction. The prodrugs were

shown to be activated by microsomal preparations expressing CYP1B1 enzyme, and to have cytotoxic activity against cell lines expressing the same enzyme, whilst having much lower cytotoxic activity against cell lines not expressing the enzyme.

5 The present invention is directed to a new class of prodrugs which are expected to be hydroxylated *in situ* by CYP enzymes, in particular enzymes expressed at high levels in tumours. In particular the prodrugs are believed to be metabolisable by CYP1B1 enzyme. Some of the compounds are new. The present invention relates to the first therapeutic use of a broad range of
10 compounds, and their synthesis as well as intermediates used therein.

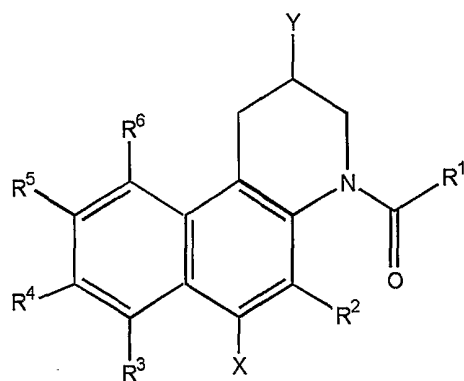
There is provided according to the first aspect of the invention the new use of a compound of the general formula I or IA or a salt thereof in the manufacture of a composition for use in a method of treatment by therapy of
15 an animal:

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I

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IA

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30 in which, X is H;

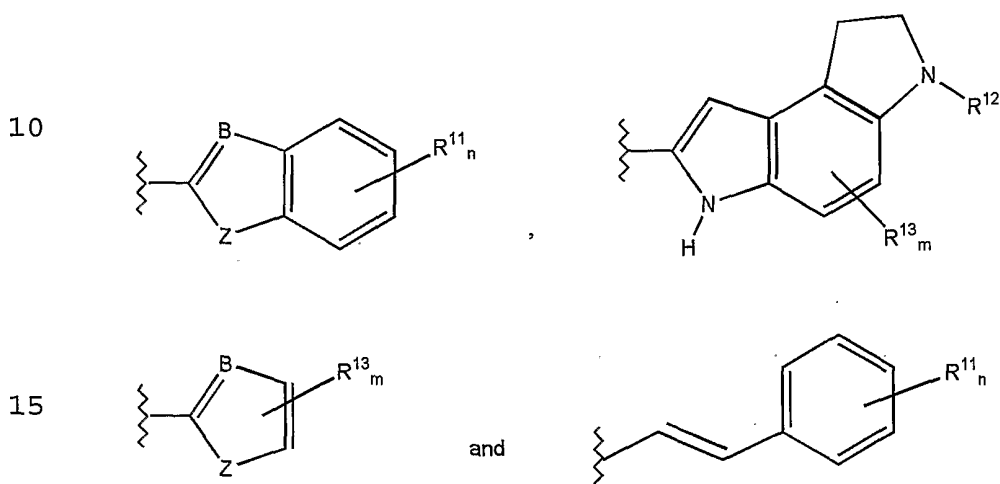
Y is a leaving group

R¹ is -Ar, NH₂, OR⁷ or R⁷;

R^2 , R^3 , R^4 , R^5 and R^6 are each independently selected from H, C_{1-4} alkyl, -OH, C_{1-4} alkoxy, -CN, Cl, Br, I, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -NHCOR⁸, -COOH, CONHR⁹, -NHCOOR⁹ and -COOR⁹;

R^7 , R^8 and R^9 are independently selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, optionally substituted heteroaryl and a ligand;

Ar is selected from



in which B is N or CR¹⁰;

R^{10} is selected from OH, C_{1-4} alkoxy, C_{1-4} alkyl, -NO₂, -NH₂, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵ and -COOR¹⁵ and H;

Z is O, S, -CH=CH- or NH;

the or each R^{11} is selected from OH, C_{1-4} alkoxy, C_{1-4} alkyl, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵ and -COOR¹⁵;

n is an integer in the range 0 to 4;

R^{12} is H, -COAr¹, -CONH₂, -COOH, -COOR¹⁵ or -COR¹⁵;

the or each R^{13} is selected from OH, C_{1-4} alkoxy, C_{1-4} alkyl, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵ and -COOR¹⁵;

m is 0, 1 or 2;

R^{14} is selected from C_{1-4} alkyl, optionally substituted phenyl, optionally substituted heteroaryl, C_{7-12} aralkyl, Ar¹ and ligands;

R^{15} is selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl optionally substituted heteroaryl and ligands;

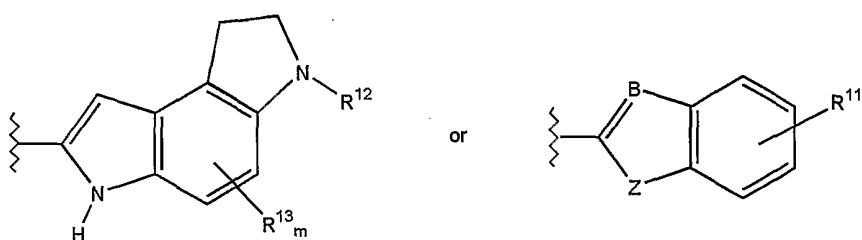
the or each R^{16} is independently selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl; and

5 Ar^1 is selected from the same groups as Ar;

provided that no more than one group R^{11} or R^{13} in any one ring includes a group Ar^1 .

Ar^1 is preferably

10



15

The animal which is treated is generally a human, although the compounds may also have veterinary use. The indication treated is generally cancer including adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, 20 ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus.

20

The tumour may, for instance, be defined as a tumour expressing high levels of CYP1B1.

25

In the invention, the leaving group Y is, for instance, in a group which has utility in nucleophilic substitution reactions. Suitable examples of leaving groups are $-OCOOR^{17}$, $-OCONHR^{18}$, Cl, Br, I, or $-OSOOR^{19}$, in which R^{17} , R^{18} and R^{19} are selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl. Most preferably the leaving 30 group is a halogen atom, preferably chlorine.

30

Optional substituents in phenyl, aralkyl and heteroaryl groups are, for instance, C_{1-4} -alkyl, halogen, hydroxyl, C_{1-4} -alkoxy, $-NH_2$, $-NHR^{16}$, $-NR^{16}_2$,

$-N^+R^{16}_3$, $-NO_2$, $-CN$, $-COOH$, $-NHCOR^{14}$, $-CONHR^{15}$, $-NHCOOR^{15}$, $-COOR^{15}$
etc.

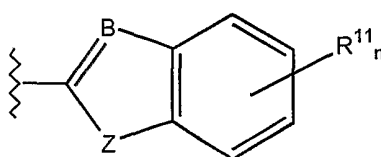
In the present invention the term ligand includes a group having specific targeting characteristics, useful for instance in antibody or gene-directed enzyme prodrug-type environments. A ligand may be an
5 oligopeptide, biotin, avidin or streptavidin, a polymeric group, an oligonucleotide or a protein. Preferably it has specific binding characteristics such as an antibody or fragment, an antigen, a sense or anti-sense oligo-nucleotide, or one of avidin, streptavidin and biotin, that is it is
10 one component of a specific binding pair. Alternatively it may be a group designed for passive targeting, such as a polymeric group, or a group designed to prolong the stability or reduce immunogenicity such as a hydrophilic group. US-A-5843937 discloses suitable ligands for conjugating to these types of actives and methods for carrying out the conjugation.

15 In a pharmaceutically active compound R^1 is other than OR^7 .

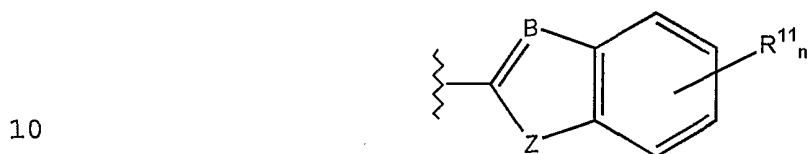
In general, for optimised DNA binding ability, the group R^1 in a compound of the general formula I is a group Ar. Often the compound may include two aromatic groups joined to one another. In such compounds, one of the groups R^{11} of the Ar group, or the group R^{12} , as the case may be, is a
20 group Ar^1 . Whilst for some compounds it may be desirable for three or more such aromatic groups to be linked, it is preferred that there is one group Ar and one group Ar^1 . Thus in a group Ar^1 which is a pyrrolo-dihydroindole type of group, the group R^{12} should be other than a group $-COAr^1$. In a group Ar^1 which is one of the other types of group there should preferably either be no
25 substituents R^{11} or R^{13} , as the case may be, or, if there are any substituents, no such substituents should include a group Ar^1 .

According to one embodiment of the invention, the substituent Ar is a group

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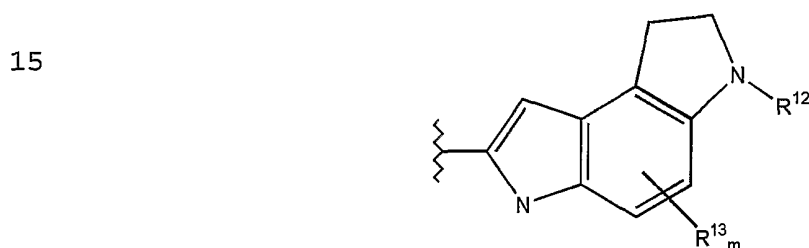


In such groups Ar, B is preferably CR¹⁰. R¹⁰ is preferably H. The definition of Z is preferably NH, although furan (Z=O) and thiophene (Z=S) analogues had been generated for conjugation to DNA alkylating units and may have useful DNA binding characteristics. Similarly, in a group Ar¹, the groups B and Z are selected amongst the same preferable groups.
 5 Preferably n is at least 1 and one of the groups R¹¹ is -NHCOAr¹. In this embodiment Ar¹ is preferably a group



in which B and Z are the same as in Ar.

In another embodiment the substituent Ar is a group



20 Preferably R¹² in Ar is other than -COOR¹⁵, more preferably it is a group -COAr¹ in which Ar¹ preferably is the same type of group.

In both groups Ar and Ar¹, m in the indole type group is preferably zero.

25 In Ar and Ar¹, there may be several substituents R¹¹. Most preferably such substituents are selected amongst C₁₋₄-alkoxy groups.

In compounds of the formula I, the core indole ring of the DNA alkylating sub-unit is preferably unsubstituted in the benzene ring (R² is hydrogen), whilst the benz ring may be unsubstituted (R³, R⁴, R⁵ and R⁶ are all hydrogen), or one or more of R³ to R⁶ represents a cyano group, an alkoxy group, a group -COOR¹⁰, or a C₁₋₄-alkyl group (preferably methyl).
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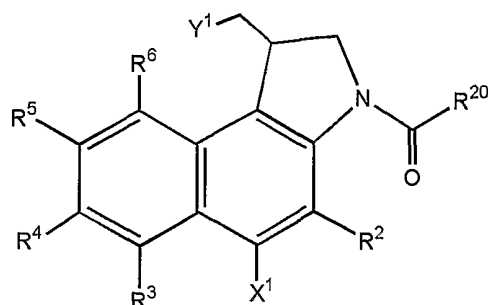
In one preferred embodiment R⁵ is a alkoxy, preferably methoxy and R², R³, R⁴, R⁶ are all H.

In another embodiment R^5 is cyano and R^2 , R^3 , R^4 and R^6 are H.

In the compounds of the formula I, X is H. It is believed that, hydroxylation of the compound will occur *in situ* at the carbon atom to which X is attached, thereby activating the compound enabling it to act as a DNA
5 alkylating agent.

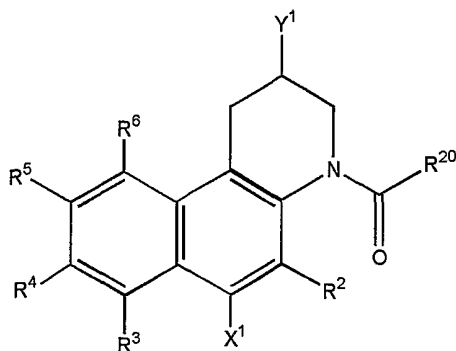
Many of the compounds of the general formula I and salts thereof are believed to be novel compounds. According to a further aspect of the invention there is provided a new compound of the general formula II or a salt thereof

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II

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IIA

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25 in which R^2 , R^3 , R^5 and R^6 are as defined above

X^1 is H;

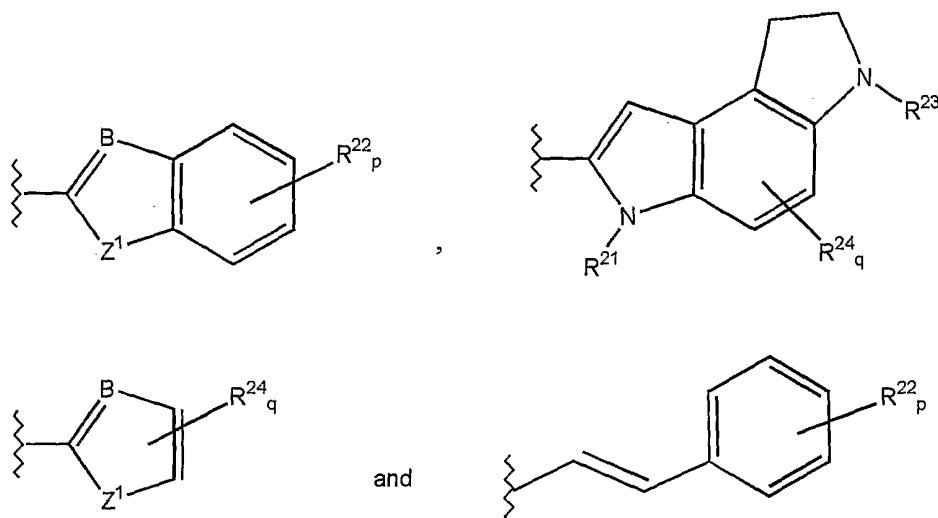
Y^1 is a leaving group;

R^{20} is $-R^7$, $-OR^7$, $-NH_2$ or Ar^2 ;

R^7 is as defined above;

30

Ar^2 is selected from



in which B¹ is N or CR²²;

Z¹ is O, S, -CH=CH- or NR²¹;

R²¹ is an amine protecting group;

5 the or each R²² is selected from OH, C₁₋₄ alkoxy C₁₋₄ alkyl, NO₂, -NHR²¹, -NHR²⁶, -NR²⁶₂, -N⁺R²⁶₃, -CN, Cl, Br, I, -NHCOR²⁵, -COOH, -CONHR⁷ and -COOR²⁵;

p is an integer in the range 0 to 4;

10 R²³ is H, COAr³, -CONH₂, -COOH, -CONHR⁷ or -COR⁷ or is an amine protecting group;

the or each R²⁴ is selected from OH, C₁₋₄ alkoxy C₁₋₄ alkyl, NO₂, -NHR²¹, -NHR²⁶, -NR²⁶₂, -N⁺R²⁶₃, -CN, Cl, Br, I, -NHCOR²⁵, -COOH, -CONHR⁷ and -COOR⁷;

q is 0, 1 or 2;

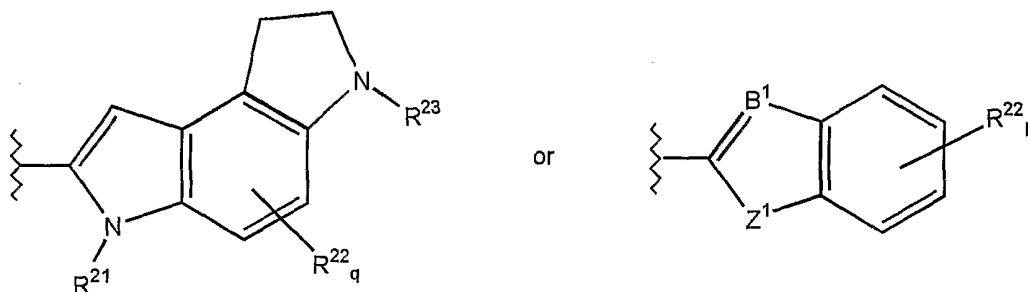
15 R²⁵ is selected from C₁₋₄ alkyl; optionally substituted phenyl, optionally substituted heteroalkyl, C₇₋₁₂ aralkyl Ar³ and a ligand.

R²⁶ is selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂ aralkyl and optionally substituted heteroaryl; and

Ar³ is selected from the same groups as Ar²

20 provided that no more than one R²² or R²⁴ in any one ring includes a group Ar³.

Ar³ is preferably



Compounds of the formula II or IIA, in which primary or secondary amine nitrogen atoms are protected are generally deprotected before being used in pharmaceutical compositions. Examples of amine protecting groups

5 R²¹ or R²³ are benzyl, benzyloxycarbonyl, tertiary butyloxycarbonyl (BOC), fluorenyl-N-methoxy-carbonyl (Fmoc) and 2-[biphenyl-(4)]-propyl-2-oxycarbonyl. Where more than one amine group is protected in the molecule, the protecting groups may be the same or different. In a particularly useful series of compounds of the general formula II and II A, R²⁰

10 is OR⁷ and R⁷ is an amine protecting group different to R¹CO. In another preferred series R²⁰ is other than OR⁷.

In compounds of the general formula II and IIA, R²⁰ is preferably Ar², and/or Y¹ is preferably selected from -OCOOR¹⁷, -OCONHR¹⁸, Cl, Br, I, or -OSOOR¹⁹, in which R¹⁷, R¹⁸ and R¹⁹ are selected from C₁₋₄ alkyl, optionally

15 substituted phenyl, C₇₋₁₂-aralkyl and optionally substituted heteroaryl. Most preferably the leaving group is a halogen atom, preferably chlorine.

The present invention further provides pharmaceutical compositions comprising compounds of the formula I or IA and a pharmaceutically acceptable excipient. Pharmaceutical compositions may be suitable for

20 intramuscular, intraperitoneal, intrapulmonary, oral or, most preferably, intravenous administration. The compositions contain suitable matrixes, for example for controlled or delayed release. The compositions may be in the form of solutions, solids, for instance powders, tablets or implants, and may comprise the compound of the formula I in solid or dissolved form. The

25 compound may be incorporated in a particulate drug delivery system, for

instance in a liquid formulation. Specific examples of suitable excipients include lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate. Solid compositions may take the form of powders and gels but are more conveniently of a formed type, for example as tablets, cachets or capsules (including spansules). Alternative, more specialised types of formulation including liposomes, nanosomes and nanoparticles.

Compounds of the formula I may be synthesised using techniques analogous to those summarised by Boger *et al* 1997, *op. cit.* It is convenient to form the DNA alkylating sub unit in one series of steps and to attach this through the nitrogen atom of the dihydro-pyrrole or tetrahydroquinoline (C) ring to the rest of the molecule. The DNA alkylating sub-unit may be conjugated to DNA binding sub-units synthesised as described in Boger *et al*, 1997 *op. cit.*, for instance the PDE-I and PDE-II sub-units described in that reference. The DNA binding subunits are those including the groups Ar and Ar¹.

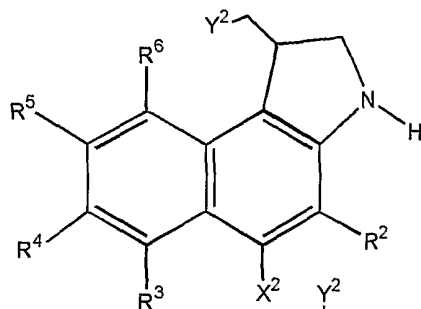
According to a further aspect of the invention there is provided a new synthetic method in which a compound of the formula III or IIIA

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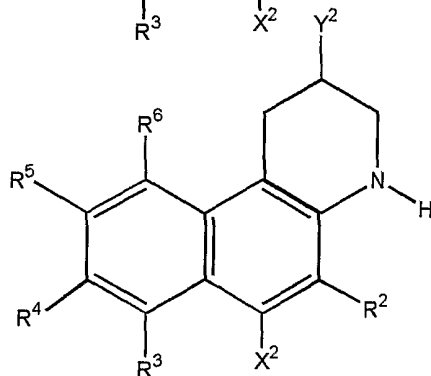
13

5



III

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IIIA

in which R², R³, R⁴, R⁵ and R⁶ are as defined above;

15

X² is H; and

Y² is a leaving group or a hydroxyl or protected hydroxyl group;

is reacted with a compound of the general formula IV

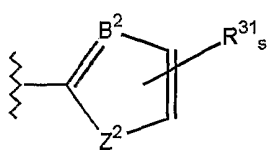
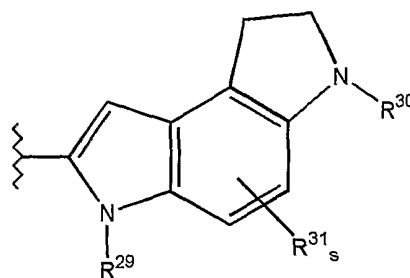
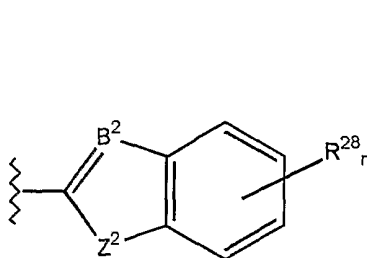


in which R²⁷ is selected from C₁₋₄-alkyl, optionally substituted phenyl,

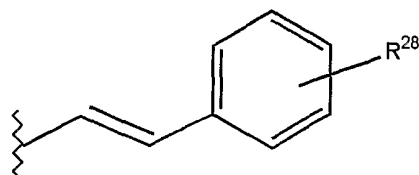
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C₇₋₁₂-aralkyl, optionally substituted heteroaryl and Ar⁴;

Ar⁴ is selected from



and



in which B^2 is N or CR^{32} ;

Z^2 is O, S, $-CH=CH-$ or NR^{33} ;

the or each R^{28} is selected from C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, I, $-NHR^{33}$, $-NHR^{35}$, $-NR^{35}_2$, $-N^+R^{35}_3$, $-NHCOR^{34}$, $-COOH$, $-CONHR^{36}$ and
 5 $-COOR^{36}$;

r is an integer in the range 0 to 4;

R^{29} is an amine protecting group;

R^{30} is an amine protecting group, $-CONH_2$, $-COOH$, $-COR^{36}$ or $-COAr^5$;

the or each R^{31} is selected from C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, I, $-NHR^{33}$, $-NHR^{35}$, $-NR^{35}_2$, $-N^+R^{35}_3$, $NHCOR^{34}$, $-COOH$, $-CONHR^{36}$ and
 10 $-COOR^{36}$;

s is 0, 1 or 2;

R^{32} is selected from H, C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, I, $-NHR^{53}$, $-NHR^{35}$, $-NHR^{35}_2$, $-N^+R^{35}_3$, $NHCOR^{34}$, $COOH$, $-CONHR^{36}$ and
 15 $COOR^{36}$;

R^{33} is an amine protecting group;

R^{34} is selected from Ar^5 , C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, optionally substituted heteroaryl and a ligand;

R^{35} is selected from C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl;
 20

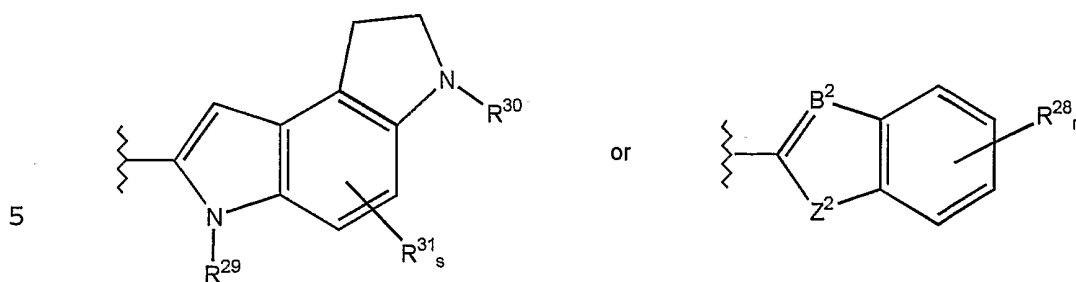
R^{36} is selected from C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl optionally substituted heteroaryl and a ligand;

Ar^5 is selected from the same groups as Ar^4 and

Y^3 is a leaving group

25 provided that no more than one R^{28} or R^{31} in any one ring includes a group Ar^5 .

A^5 is preferably



10 Y^3 is, for instance, selected amongst the preferred leaving groups listed above for Y. Most suitably the definition of Y^3 is Cl. Alternatively, the group Y^3 may be OH. In this case, it may be necessary to include a coupling agent to assist in the coupling reaction.

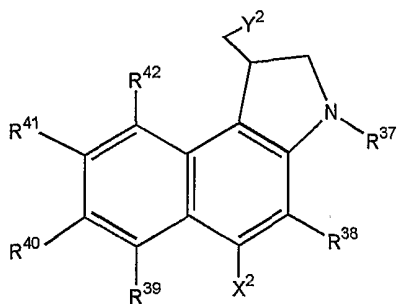
15 The reaction between the compound of the general formula III or IIIA and the carboxylic acid or derivative of the general formula IV is carried out under conditions allowing such coupling to take place. Such conditions are similar to those generally used for formation of peptide bonds, for instance as used in peptide synthetic methods.

Y^2 is a hydroxy or protected hydroxyl group or a leaving group, which may be the same as Y, or may be converted to Y in a subsequent step.

20 After the coupling process, it may be desirable to deprotect one or more of the protected amine groups. If further reaction, for instance with other derivatising agents such as glycosyl compounds, peptides, polymers etc is desired through any such amine groups, it may be desirable to deprotect only those to which subsequent reaction to take place, whilst retaining the other amine groups in a protected form. Selection of suitable amine protecting groups and protection and deprotection protocols may be made using techniques commonly utilised in peptide chemistry.

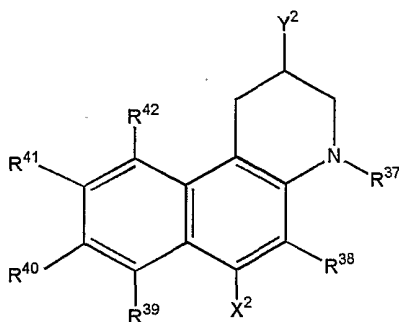
25 It is believed that some of the intermediates of the general formula III or IIIA may be novel compounds. A novel compound may have the general formula V or VA

5



V

10



VA

15

in which R^{38} , R^{39} , R^{40} , R^{41} and R^{42} are selected from H, C_{1-4} -alkyl, -OH, C_{1-4} -alkoxy, -CN, Cl, Br, I, NO_2 , NHR^{43} , $-NHCOR^{44}$, $-NR^{45}_2$, $-N^+R^{45}_3$, -COOH, -CONHR⁴⁶ and -COOR⁴⁶

X^2 is H;

Y^2 is a leaving group or a hydroxyl or protected hydroxyl group;

R^{37} is H or an amine protecting group;

20

R^{43} is selected from H, C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, and optionally substituted heteroaryl an amine protecting group;

R^{44} is selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, optionally substituted heteroaryl and a ligand;

25

each R^{45} is selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl; and

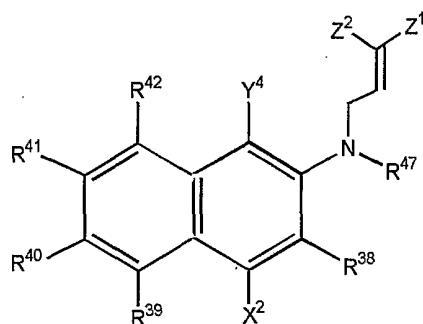
R^{46} is selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, optionally substituted heteroaryl and a ligand.

30

In compounds of the general formula III, in the compound ready for reaction with a carboxylic acid derivative, for instance of the general formula IV, R^{24} is H. Precursors for such compounds have the ring nitrogen atom in protected form, that is in which R^{37} represents a protecting group.

In compounds of the formula V, the group Y^2 may be selected amongst those defined above for leaving group Y. The nature of the group Y^2 should be selected having regard to the nature of the reagent with which the compound of the formula III/IV is to react in a subsequent step, for instance with a compound of the general formula IV, such that the group Y^2 is not deactivated and does not form a dimer of compounds of the formula III or V. Suitable examples of leaving group Y^2 are Cl and Br.

The compound of the formula III or IIIA and V or VA may be prepared in a preliminary step including a cyclisation step in the presence of a catalyst using as the starting material an aniline compound having a leaving group substituent Y^4 at the carbon atom ortho to the amine group substituent, and an N-substituent which is a group $-CH_2CH=CHY^5$, in which the aniline derivative is reacted under cyclisation conditions, to form a dihydropyrrole or a di- or tetra hydroquinoline ring. The starting compound for such a reaction may be represented by the general formula VI



VI

in which R^{38} through R^{42} and X^2 are the same as in the compound of the formula IV;

- 25 R^{47} is an amine protecting group,
 one of Z^1 and Z^2 is Y^5 and the other is H;
 Y^5 is a leaving group which is different from or the same as Y^2 ; and
 Y^4 is the radical leaving group.

30 For cyclisation to form a dihydropyrrole ring, the group Z^1 is Y^5 and Y^5 is either H or a leaving group, preferably the same group as Y^2 wherein the group Y^5 is not effective as a leaving group in this step of the synthesis. The reaction is conducted in the presence of a suitable catalyst, optionally in the

presence of a free radical trap. The group Y^4 should be a radical leaving group, such as halogen, preferably Br or I. Suitable radicals for carrying out the cyclisation reaction using a compound VI in which Y^5 is H are nitroxyl compounds such as 2,2,6,6-tetramethylpiperidinyloxy (TEMPO). Where Y^5 ,
5 is a leaving group the cyclisation may be carried out in the presence of a radical derived from azoisobutyronitrile (AIBN). Suitable catalysts for such a radical cyclisation step are tin hydride compounds such as tributyl tin hydride. Such a synthetic route is illustrated in Examples 1 and 3.

For cyclisation to form a 6-membered ring it is preferred to use a
10 compound VI in which Z^2 is Y^5 and Y^5 is a leaving group, preferably a trialkyl stannyl group, and to carry out the reaction in the presence of a suitable catalyst palladium complexes such as tetrakis (triphenylphosphine) palladium (0), bis(triphenyl phosphine) palladium (II) chloride or palladium (II) acetate. The dihydroquinoline intermediate is oxidised to form a further
15 intermediate which is an epoxide, for instance using a peroxide reagent. The epoxide intermediate is reduced using a suitable selective reducing agent such as a dialkyl aluminium hydride to produce the corresponding tetrahydroquinoline alcohol which is subsequently halogenated, for instance using carbon tetrachloride/triphenyl phosphine. This reaction is illustrated in
20 Example 2.

The compound of the general formula VI may be produced by alkylation of the sodium salt of the corresponding aniline derivative with a 1,3-dihalo propene compound.

The carboxylic acid derivative of the general formula V may be
25 synthesised using the methods generally described in Boger *et al*, 1997 *op.cit.* for instance PDE-I and PDE-II may be synthesised using the Umezawa synthesis, the Rees-Moody synthesis, the Magnus synthesis, the Cava-Rawal synthesis, the Boger-Coleman synthesis, the Sundberg synthesis, the Martin synthesis, the Tojo synthesis. Indole-2-carboxylic acid
30 is commercially available. Other analogues of the DNA binding sub-units of the duocarmycins, and reactive carboxylic acid derivatives thereof are described by Boger *et al*, *op.cit.* and in US-A-5843937.

The present invention relates to the creation of a range of prodrugs that have little or no cytotoxic effects when in their normal state, but are highly cytotoxic (i.e. have a substantially increased cytotoxicity) when activated by oxidation or hydroxylation by CYP enzymes. This provides for a self-targeting drug delivery system in which a non cytotoxic (or negligibly cytotoxic) compound can be administered to a patient, for example in a systemic manner, the compound then being activated at the site of the tumour cells (intratumoural activation) to form a highly cytotoxic compound which acts to kill the tumour cells. The fact that the CYP isoforms are not expressed by normal cells mean that the activation of the compound only occurs at the site of the tumour cells and therefore only tumour cells are affected, thus providing a self-targeting system.

The prodrugs of the present invention have the distinct advantage of being useful in the treatment of tumours at any site in the body, meaning that even tumours that have undergone metastasis (which are normally not susceptible to site specific therapies) may be treated.

The prodrug may be an antitumour prodrug. Examples of tumours include cancers (malignant neoplasms) as well as other neoplasms e.g. innocent tumours. The prodrug may be activated by hydroxylation by isoforms of cytochrome P450's.

In a variation of the normal procedure which relies upon CYP expression within tumour cells to effect selective hydroxylation and hence activation of the prodrugs, the selectivity between tumour tissue and normal tissue can be enhanced in a two part procedure. Thus (a) infecting tumor cells with a viral vector carrying a cytochrome P450 gene and a cytochrome P450 reductase gene, wherein expression of cytochrome P450 gene and cytochrome P450 reductase gene by tumor cells enables the enzymatic conversion of a chemotherapeutic agent to its cytotoxic form within the tumor, whereby the tumor cells become selectively sensitized to the prodrug chemotherapeutic agent (b) contacting tumor cells with the prodrug chemotherapeutic agent whereby tumor cells are selectively killed.

These prodrugs are benz(e)dihydroindole or benz-tetrahydroquinoline derivatives. Their specific use as antitumour prodrugs has not been previously suggested or disclosed, nor has the suggestion that they are prodrugs having an activated hydroxylated form. Where compounds of formula (I) have been previously identified and made, they have not been identified as anti-tumour agents due to their poor (or negligible) cytotoxicity. Thus the intratumoural hydroxylation of the prodrugs of the present invention provides them with a surprising and unexpected efficacy.

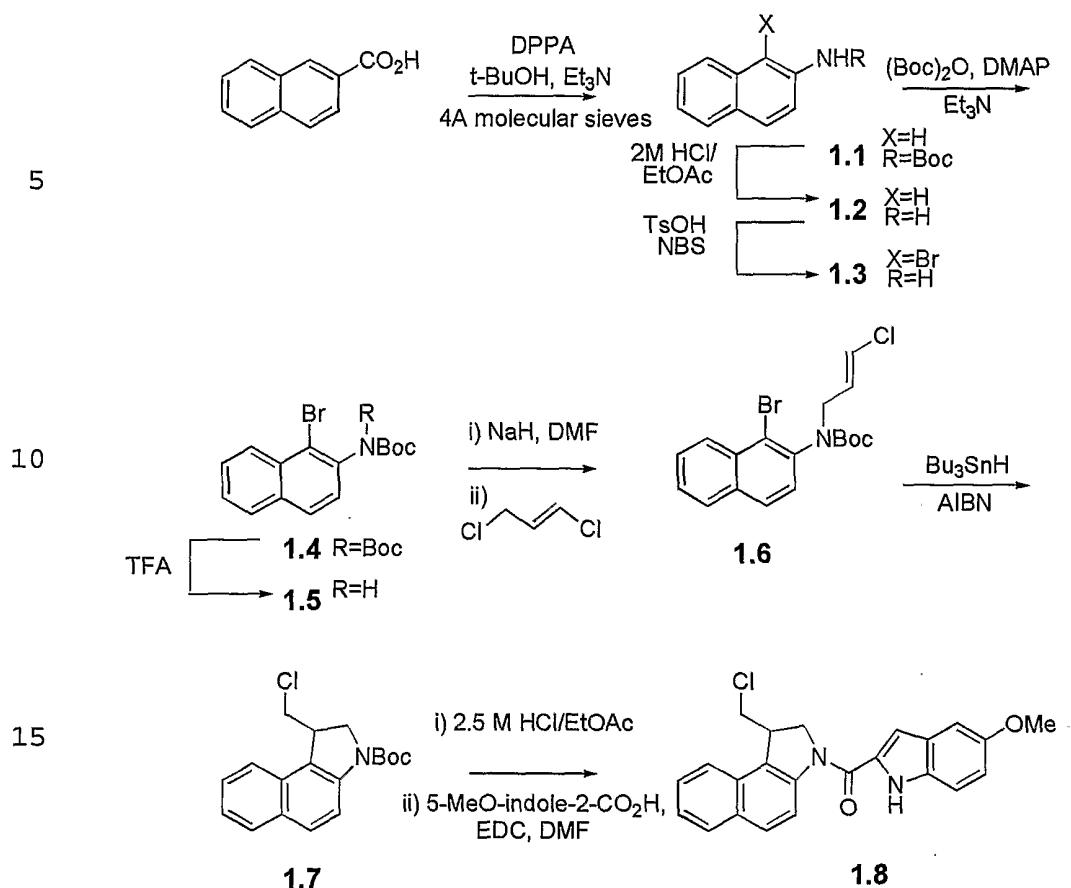
Hydroxylated forms of the prodrugs are potent DNA alkylating agents that bind in the minor groove of DNA and alkylate the purine bases at the N3 position. As such, they are potent cytotoxic agents whose exact biological mechanism of action is unknown but involves the disruption of template and other functions of DNA. General inhibition of template function of DNA will affect and be generally cytotoxic to all dividing cells in the body and lead to unacceptable side effects in a therapeutic setting. However, the targeted production of hydroxylated forms only in tumour cells that overexpress particular isoforms of cytochrome P450's will lead to a specific cytotoxic effect only in those cells. The non-hydroxylated forms are essentially non-toxic to all cells.

The following examples illustrate the invention.

25

30

Example 1

20 **1.1 N-(*tert*-Butyloxycarbonyl)naphthylamine**

A solution of 2-naphthoic acid (100 mg, 0.581 mmol) in t-BuOH (33 mL) was treated with Et₃N (96 μL, 0.7 mmol) and 4 Å molecular sieves (1 g). Diphenyl phosphorylazide (0.15 ml, 0.7 mmol) was added, and the reaction mixture was warmed to reflux for 14 h. The mixture was cooled to 25 °C and the solvent removed under vacuum. The residue was dissolved in EtOAc (10 mL) and the organic phase was washed with 10% aqueous HCl (3 x 15 mL), dried (MgSO₄) and concentrated. Purification by flash chromatography (SiO₂, 10 % EtOAc in hexanes) afforded **2** (0.108mg, 77%) as a pale yellow solid. FABMS (NBA/NaI) 243([M + H]⁺ expected 243) 269 ([M + Na]⁺ expected 269).

25

30

1.2 2-Naphthylamine

Compound **1.1** (1.5 g, 6.17 mmol) was dissolved in EtOAc (10 mL) to which was added 2.5 M HCl in EtOAc. The reaction mixture was stirred for 30 min. The resulting solution was then diluted with sat'd aq. NaHCO₃ (50 mL) and
5 extracted with EtOAc (2 x 25 mL). The combined organic layers were dried (MgSO₄) and concentrated. The residue was crystallised from 20% EtOAc in hexane to afford **3.3** (0.74g, 84%) as pale brown crystals: ¹H NMR (250 MHz, CDCl₃) δ ppm 7.12-7.32 (m, 3 H), 6.96 (t, 1 H), 6.80 (t, 1 H), 6.56 (s, 1 H), 6.52 (dd, 1 H), 3.46 (br s, 2 H); FAB MS (NBA/NaI) 143 ([M + H]⁺
10 expected 143), 165 ([M + Na]⁺ expected 165).

1.3 1-Bromo-2-naphthylamine

A solution of **1.2** (100 mg, 0.7 mmol), TsOH (48 mg, 0.28 mmol) in THF (6 mL) was stirred and cooled to 0°C. To the resulting mixture NIS (125 mg, 0.56 mmol) and THF (6 mL) were added and the solution was allowed to
15 warm to 25 °C. After 3 h, additional NIS (31 mg, 0.14 mmol) was added. After 1 h, the mixture was diluted with 10% aqueous NaHCO₃ (10 mL) and extracted with CHCl₃ (3 x 10 mL). The organic layers were combined, dried (MgSO₄) and concentrated. The residue was purified by flash
chromatography (SiO₂, 30 % EtOAc in hexane) to afford **1.3** (110 mg, 59 %) as a brown-yellow oil ¹H NMR (250 MHz, CDCl₃) δ ppm 8.04 (d, 1 H), 7.68 (d,
20 1 H), 7.62 (d, 1 H), 7.50 (t, 1 H), 7.28 (t, 1 H), 7.00 (d, 1 H), 4.40 (br s, 2 H); FABMS (NBA/NaI): 221 ([M + H]⁺ expected 221), 243 ([M + Na]⁺ expected 243).

1.4 N-Di(*tert*-butyloxycarbonyl)-1-bromo-2-naphthylamine

A solution of **1.3** (100 mg, 0.37 mmol) in CH₂Cl₂ (5mL) was treated with Boc-dicarbonate (219 mg, 1.0 mmol), Et₃N (62 μl, 0.45 mmol) and DMAP (4.5 mg, 0.037 mmol). The reaction was refluxed at 50 °C for 24 h. The resulting
25 mixture was washed with H₂O (2 x 10 mL), 5% HCl (10 mL) and finally again with H₂O (10 mL). The organic layers were combined, dried (MgSO₄) and
30 concentrated. The reaction was purified by flash chromatography (SiO₂ CH₂Cl₂/hexane 1:1) to afford **1.4** (125 mg, 72%) as a colourless solid. ¹H

NMR (250 MHz, CDCl_3) δ ppm 8.32 (d, 1 H), 7.86 (d, 1 H), 7.80 (d, 1 H), 7.52-7.68 (m, 2 H), 7.32 (d, 1 H), 1.50 (s, 18 H); FABMS (NBA/NaI) 422 ($[\text{M} + \text{H}]^+$ expected 422), 446 ($[\text{M} + \text{Na}]^+$ expected 446).

1.5 *N*-(*tert*-Butyloxycarbonyl)-1-bromo-2-naphthylamine

5 Compound **1.4** (50mg, 0.107 mmol) was treated with a solution of TFA (16 μL , 0.213 mmol) in CH_2Cl_2 (2mL) and stirred for 45 min. The mixture was concentrated and recrystallised from hexane to afford **1.5** (13 mg, 34 %) as white crystals. FABMS (NBA/NaI) 323 ($[\text{M} + \text{H}]^+$ expected 323), 346 ($[\text{M} + \text{Na}]^+$ expected 346).

10 **1.6 *N*-(*tert*-Butyloxycarbonyl)-*N*-(3-chloro-2-propen-1-yl)-1-bromo-2-naphthylamine**

A solution of **1.5** (50 mg, 0.14 mmol) and DMF (5 mL) was cooled to 0 °C and NaH (9.8 mg, 0.408 mmol) was added. The resulting mixture was stirred for 15 min and 1,3 dichloropropene (38 μL , 0.41 mmol) was added. The
15 solution was allowed to warm to 25 °C and stirred for 90 min. The mixture was concentrated and the residue was purified by flash chromatography (SiO_2 , 10 % EtOAc in hexane) to afford **1.6** (56 mg, 93 %) as a clear film. FABMS (NBA/NaI) 396 ($[\text{M} + \text{H}]^+$ expected 396), 418 ($[\text{M} + \text{Na}]^+$ expected 418).

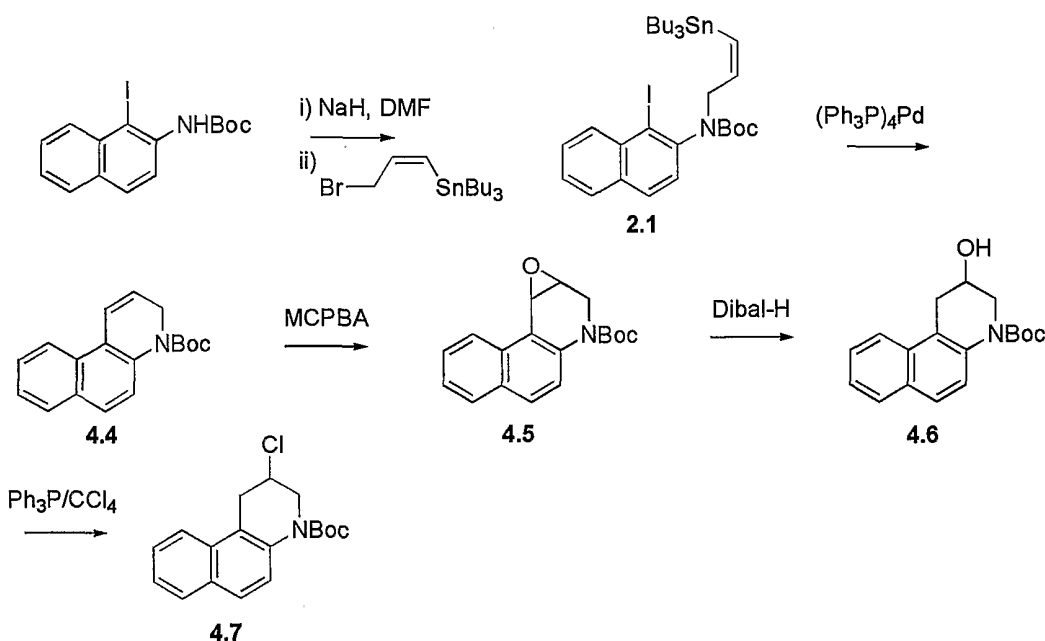
20 **1.7 3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-benz[e]indole**

A solution of **1.6** (55 mg, 0.12 mmol) and AIBN (8 mg, 0.05 mmol) in anhydrous toluene (5 mL) was degassed for 15 min with N_2 and then heated to 90 °C. Bu_3SnH (65 μl , 0.25 mmol) was added in four portions over 1 h and
25 the resulting mixture was stirred for 1 h. The solution was then concentrated and purified by flash chromatography (SiO_2 , 10% EtOAc in hexane) to afford impure **3.8**. The solid was then dissolved in EtOAc (2 mL) to which a 1M KF solution (1ml) in EtOAc (1ml) was added. The solution was stirred for 45 mins after which the insoluble precipitate was filtered and the remaining
30 solution concentrated to afford pure **1.7** (34mg, 87%) as a pale yellow oily film. FABMS (NBA/NaI) 317 ($[\text{M} + \text{H}]^+$ expected 317).

1.8 1-(Chloromethyl)-3-[(5-methoxyindol-2-yl)carbonyl]-1,2-dihydro-3H-benz[e]indole

Compound **1.7** (10 mg, 0.03 mmol) was treated with 2.5 M HCl in EtOAc (100 μ L) and the solution was stirred for 30 min. The solvent was removed under a stream of nitrogen and the grey residue was dissolved in DMF (1 mL). 5-Methoxyindole-2-carboxylic acid (17 mg, 0.09 mmol) and EDC (17 mg, 0.09 mmol) were added and the mixture stirred for 16 h. Solvent was removed *in vacuo* and the residue subjected to flash chromatography (SiO₂, EtOAc/hexanes 1:1) to give the product as a red oil (11 mg, 94 %). FABMS (NBA/NaI) 391 ($M + H^+$ expected 391).

Example 2



2.1 2-[N-(3-(tributylstannyl)-2-propen-1-yl)-N-((tert-butyloxy)carbonyl)]amino-1-naphthalene

1-benzoyl-5-(tert-butyloxycarbonyl)amino-4-iodoindole (100 mg, 0.22 mmol) was stirred in DMF (1 mL) and sodium hydride (26 mg, 0.66 mmol, 60% dispersion in oil, 3 equiv.) was added. After 15 min, the suspension was treated with E/Z-1-tributylstannyl-3-bromopropene (270 mg, 0.66 mmol, 3

equiv) and the resulting solution was stirred at RT for 16 h. The solution was concentrated and water (10 mL) was added. The aqueous solution was extracted with ethyl acetate (3 x 10 mL), the organic layers combined, dried and concentrated. Flash chromatography (SiO₂, 10% ethyl acetate/hexanes) gave the product (120 mg, 78%) as a yellow solid. FABMS (NBA/NaI): 699 (M + H⁺ expected 699).

2.2 1,2-dihydro-1-((*tert*-butyloxy)carbonyl)-5,6-benzoquinoline

1-Benzoyl-5-[N-(3-(tributylstannyl)-2-propen-1-yl)-N-((*tert*-butyloxy)carbonyl)] amino-4-iodoindole (100 mg, 0.14 mmol) and tetrakis(triphenylphosphine) palladium(0) (32 mg, 0.2 equiv) were stirred in toluene (2 mL) at 50°C under N₂ for 4 h. The solvent was then removed *in vacuo*. Chromatography (SiO₂, 10% ethyl acetate/hexanes) gave the product as a red oil (38 mg, 99%). FABMS (NBA/NaI): 282 (M + H⁺ expected 282).

2.3 3,4-epoxy-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline

1,2-dihydro-1-((*tert*-butyloxy)carbonyl)-5,6-benzoquinoline (100 mg, 0.36 mmol) and MCPBA (91 mg, 0.54 mmol, 1.5 equiv) were stirred in CH₂Cl₂ (2 mL) at -30 °C under N₂ for 6 h. The solvent was then removed *in vacuo*. Chromatography (SiO₂, 10% ethyl acetate/hexanes) gave the product (101 mg, 95%). FABMS (NBA/NaI): 298 (M + H⁺ expected 298).

2.4 4-hydroxy-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline

3,4-epoxy-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline. (100 mg, 0.34 mmol) was treated with Dibal-H (91 mg, 0.54 mmol, 1.5 equiv) in THF (2 mL) at -78 °C to -30 °C under N₂. After 1 h, the reaction was quenched by the addition of water (2 mL) and the resulting solution was extracted with ethyl acetate (3 x 10 mL), the organic layers combined, dried and concentrated. The solvent was removed *in vacuo*. Chromatography (SiO₂, 10% ethyl acetate/hexanes) gave the product (55 mg, 54 %) as a colourless solid. FABMS (NBA/NaI): 300 (M + H⁺ expected 300), 322 (M + Na⁺ expected 322).

2.5 4-Chloro-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline

4-hydroxy-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline (100 mg, 0.33 mmol) in CH₂Cl₂ (2 mL) was treated with a prepared solution of PPh₃ (175 mg, 0.66 mmol, 2 equiv) and CCl₄ (200 μL) in CH₂Cl₂ (2 mL) at RT. After 4 h, the solvent was removed *in vacuo*. Chromatography (Silica gel, 2 x 15 cm, 10% ethyl acetate/hexanes) gave the chloride as an oil (65mg, 63%). FABMS (NBA/NaI): 318 (M + H⁺ expected 318), 340 (M + Na⁺ expected 340). The product may subsequently be deprotected and conjugated to a DNA-binding subunit such as 5-methoxyindole-2-carboxylic acid by process steps analogous to example 1.8.

Example 3 Biological testing of compound 1.8

Materials and Methods

3.1 Incubation mixtures of test compound and microsomes

Test compound activation by CYP enzymes was carried out using NADPH supplemented rat liver microsomes. Incubation mixtures comprised microsomal protein (1 mg/ml), reduced-nicotinamide adenine dinucleotide phosphate (NADPH, 10mM) and phosphate buffer (pH7.4, 100mM). Test compound (0.01– 100 μM final concentration) in DMSO (20μl) was added to the microsomal incubation mixtures (0.5ml) and incubated for 60 min at 37C. Control incubates contained test compound and microsomal incubation mixture terminated at 0 time. All incubations were terminated by addition of an equal volume of ice-cold acetonitrile and microfuged for 3 min. Aliquots of the supernatant were added to cells in culture and cytotoxicity determined as described below.

3.2 Cell culture based cytotoxicity measurement

Chinese Hamster Ovary (CHO) cell were grown in MEM supplemented with 10% dialysed FBS and G418 (400μg/ml). All cells were seeded at an initial density of 1000 cells/well in 96-well-plates, incubation at 37°C for 24 hours. Aliquots (0.1ml) of the test compound/microsomal/acetonitrile supernatant

was then added to the CHO cells. Cells were then incubated for 24 hours at 37°C, 5% CO₂. After this time period MTT (50 µl; 2mg/ml stock solution) was added to each well and cells were incubated for a further 4 hours. During this time period MTT, a hydrogen acceptor tetrazolium salt, is reduced to formazan dye by mitochondrial dehydrogenase of viable cells. The media was aspirated from cells and DMSO (100 µl/well) added to solubilise the coloured formazan dye. Absorbance of the formazan dye in the 96-well-plates was then determined at 550nm. The effect of microsomal activation by the test compound on the arrest of CHO cell growth could be determined by comparing the IC₅₀ (concentration that inhibited cell growth by 50%) with and without microsomal incubation.

Results

15

compound	CHO IC ₅₀ (µM)		AF
	+activation	-activation	
1.8	0.1±1.2	1.5±0.53	15.0*

Effect of compound **3.9** and its metabolism (activation) product on the survival of Chinese hamster ovary cells in culture. Cells were incubated for 24 hours with supernatants from reaction mixtures of compound **3.9** with NADPH fortified rat liver microsomes. IC₅₀ represents the concentration of drug required to inhibit cell growth by 50%. Values are expressed as the mean + sd for three experiments. See methods for full details of metabolism. AF = activity factor i.e. the ratio of IC₅₀ cytotoxicity values obtained for + compound **1.8** activation

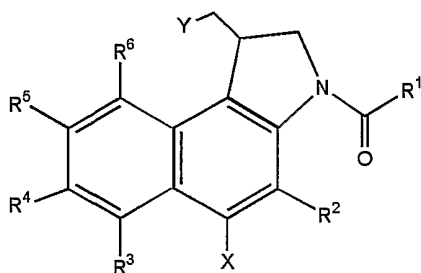
* represents significance at p>0.05.

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CLAIMS

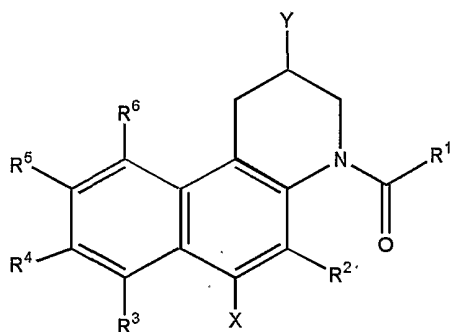
1. Use of a compound of the general formula I or IA or a salt thereof in the manufacture of a composition for use in a method of treatment by therapy of an animal:

5



I

10



IA

15

in which X is H;

20

Y is a leaving group;

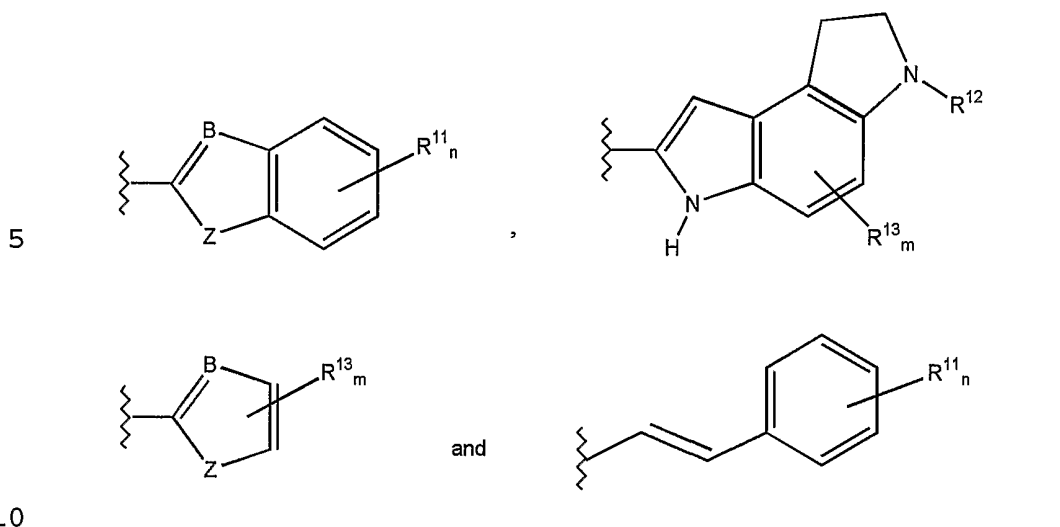
R¹ is -Ar, NH₂, R⁷ or -OR⁷;

R², R³, R⁴, R⁵ and R⁶ are each independently selected from H, C₁₋₄ alkyl, -OH, C₁₋₄ alkoxy, -CN, Cl, Br, I, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -NHCOR⁸, -COOH, -CONHR⁹, -NHCOOR⁹ and -COOR⁹;

25

R⁷, R⁸ and R⁹ are independently selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-aralkyl, optionally substituted heteroaryl and a ligand;

Ar is selected from



in which B is N or CR¹⁰;

R¹⁰ is selected from OH, C₁₋₄ alkoxy, C₁₋₄ alkyl, -NO₂, -NH₂, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵, -COOR¹⁵ and H;

15 Z is O, S, -CH=CH- or -NH;

the or each R¹¹ is selected from OH, C₁₋₄ alkoxy, C₁₋₄ alkyl, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵ and COOR¹⁵;

n is an integer in the range 0 to 4;

20 R¹² is H, -COAr¹, -CONH₂, -COOH, -COR¹⁵ or -COOR¹⁵;

the or each R¹³ is selected from OH, C₁₋₄ alkoxy, C₁₋₄ alkyl, -NO₂, -NH₂, -NHR¹⁶, -NR¹⁶₂, -N⁺R¹⁶₃, -CN, Cl, Br, I, -NHCOR¹⁴, -COOH, -CONHR¹⁵, -NHCOOR¹⁵ and -COOR¹⁵;

m is 0, 1 or 2;

25 R¹⁴ is selected from C₁₋₄ alkyl, optionally substituted phenyl, optionally substituted heteroaryl, C₇₋₁₂ aralkyl, Ar¹ and a ligand;

R¹⁵ is selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-aralkyl and optionally substituted heteroaryl and a ligand;

30 the or each R¹⁶ is independently selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-aralkyl and optionally substituted heteroaryl; and

Ar¹ is selected from the same groups as Ar;

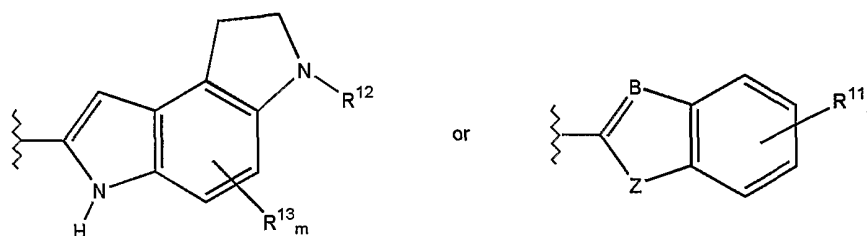
provided that no more than one group R^{11} or R^{13} in any one ring includes a group Ar^1 .

2. Use according to claim 1 in which the animal is a human.

3. Use according to claim 1 or claim 2 in which the treatment is of
5 a tumour.

4. Use according to any preceding claim in which Y is selected from $-OCOOR^{17}$, $-OCONHR^{18}$, Cl, Br, I, and $-OSOOR^{19}$, in which R^{17} , R^{18} and R^{19} are selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl; preferably Cl.

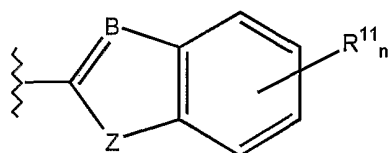
10 5. Use according to any preceding claim in which Ar^1 is



6. Use according to any preceding claim in which R^1 is Ar.

7. Use according to claim 6 in which Ar is a group

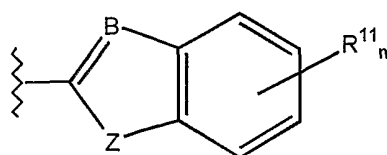
20



8. Use according to claim 7 in which n is at least one and one of
25 the groups R^{11} of the Ar group is $-NHCOAr^1$.

9. Use according to claim 8 in which Ar^1 is a group

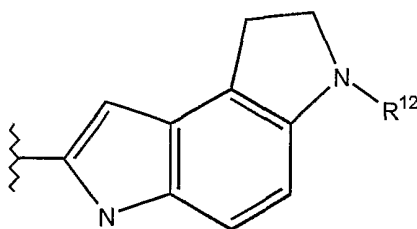
30



10. Use according to claim 9 in which, in Ar^1 , n is at least 1 and R^{11} is other than $-NHCOAr^1$, or n is 0.

11. Use according to claim 6 in which Ar is a group

5

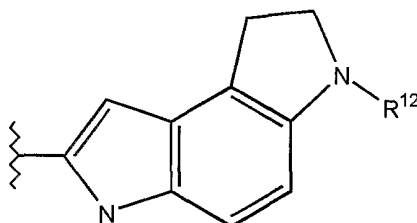


12. Use according to claim 11 in which R¹² is -COAr¹.

10

13. Use according to claim 12 in which Ar¹ is a group

15



14. Use according to claim 13 in which, in Ar¹, R¹² is other than -COAr¹.

20

15. Use according to any preceding claim in which R² is H.

16. Use according to any preceding claim in which R³ is H.

17. Use according to any preceding claim in which R⁴ is H.

18. Use according to any preceding claim in which R⁵ is H, C₁₋₄-alkoxy or -CN, preferably MeO- or H.

25

19. Use according to any preceding claim in which R⁶ is H.

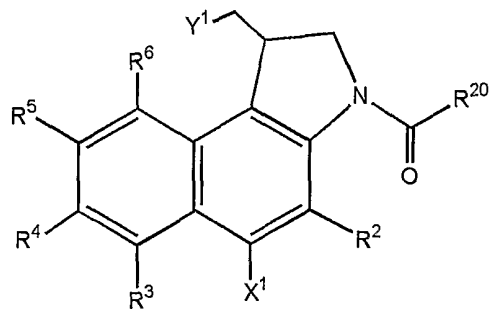
20. A compound of the general formula I as defined in any of claims 1 and 4 to 19 for use in the treatment an animal by therapy.

21. A pharmaceutical composition comprising a compound of the general formula I as defined in any of claims 1 and 4 to 19 and a pharmaceutically acceptable excipient.

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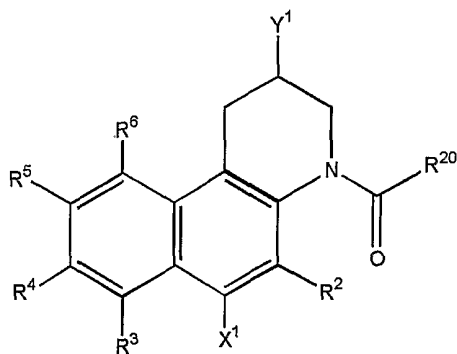
22. A compound of the general formula II or IIA or a salt thereof

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II

10



IIA

15 in which R², R³, R⁵ and R⁶ are as defined above

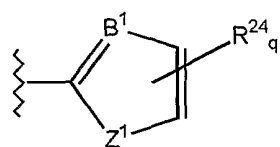
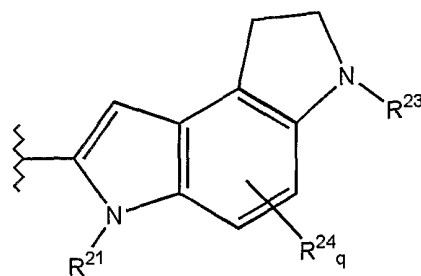
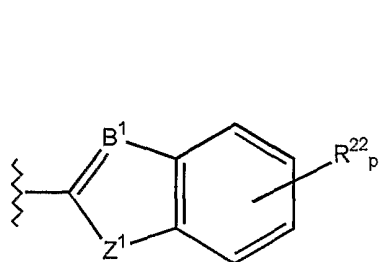
X¹ is H;

Y¹ is a leaving group;

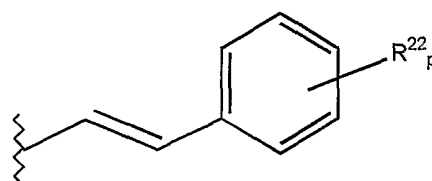
R²⁰ is R⁷, OR⁷, -NH₂ or Ar²;

20 R⁷ is selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-
aralkyl, optionally substituted heteroaryl and a ligand;

Ar² is selected from



and



in which B¹ is N or CR²²;

Z¹ is O, S, -CH=CH- or NR²¹;

R²¹ is an amine protecting group;

the or each R²² is selected from OH, C₁₋₄ alkoxy C₁₋₄ alkyl, NO₂,

5 -NHR²¹, -NHR²⁶, -NR²⁶₂, -N⁺R²⁶₃, -CN, Cl, Br, I, -NHCOR²⁵, -COOH, -
CONHR⁷ and -COOR⁷;

p is an integer in the range 0 to 4;

R²³ is H, COAr³, -CONH₂, -COOH or -COR⁷ or is an amine protecting
group;

10 the or each R²⁴ is selected from OH, C₁₋₄ alkoxy C₁₋₄ alkyl, NO₂,
-NHR²¹, -NHR²⁶, -NR²⁶₂, -N⁺R²⁶₃, -CN, Cl, Br, I, -NHCOR²⁵, -COOH, -
CONHR⁷ and -COOR⁷;

q is 0, 1 or 2

15 R²⁵ is selected from C₁₋₄ alkyl, optionally substituted phenyl, optionally
substituted heteroalkyl, C₇₋₁₂ aralkyl, Ar³ and a ligand;

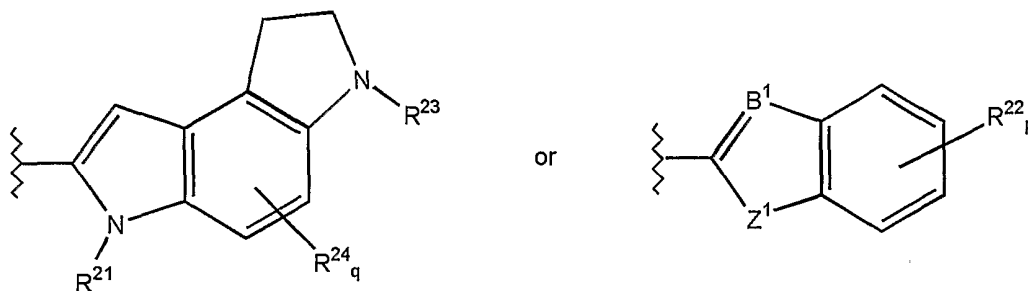
R²⁶ is selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-
aralkyl and optionally substituted heteroaryl; and

Ar³ is selected from the same groups as Ar²

20 provided that no more than one R²² or R²⁴ in any one ring includes a
group Ar³.

23. A compound according to claim 22 in which Y¹ is selected from
-OCOOR¹⁷, -OCONHR¹⁸, Cl, Br, I, and -OSOOR¹⁹ in which R¹⁷, R¹⁸ and R¹⁹
are selected from C₁₋₄ alkyl, optionally substituted phenyl, C₇₋₁₂-aralkyl and
optionally substituted heteroaryl, preferably Cl.

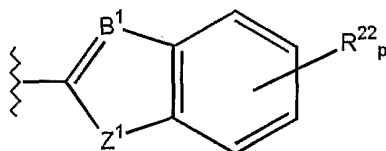
25 24. A compound according to claim 22 or claim 23 in which Ar³ is



25. A compound according to any of claims 22 to 23 in which R^{20} is Ar^2 .

26. A compound according to claim 25 in which Ar^2 is a group

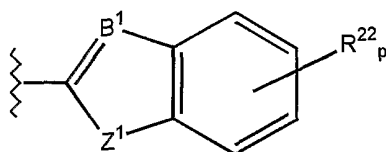
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27. A compound according to claim 26 in which p is at least 1 and one of the groups R^{22} of the group R^{20} is a group $-NHCOAr^3$.

10

28. A compound according to claim 27 in which Ar^3 is

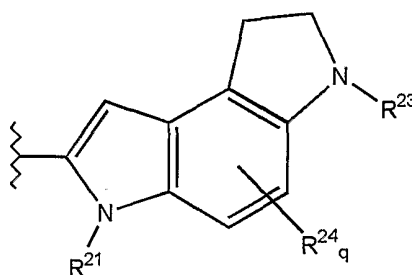


15

29. A compound according to claim 28 in which, in Ar^3 , p is at least 1 and R^{22} is other than $-NHCOAr^3$, or p is 0.

30. A compound according to claim 25 in which Ar^2 is a group

20



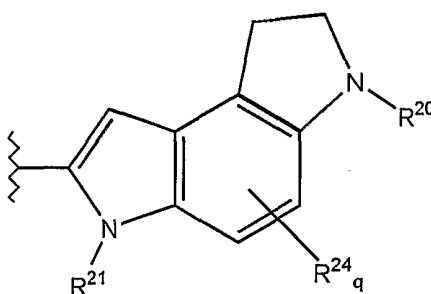
25

31. A compound according to claim 30 in which R^{23} is $COAr^3$.

32. A compound according to claim 31 in which Ar^3 is

30

35



5

33. A compound according to claim 32 in which, in Ar³, R²³ is other than -COAr³.

10 34. A compound according to claim 22 or claim 23 in which R²⁰ is other than Ar².

35. A compound according to any of claims 22 to 34 in which R¹ is H.

36. A compound according to any of claims 22 to 35 in which R³ is H.

15 37. A compound according to any of claims 22 to 36 in which R⁴ is H.

38. A compound according to any of claims 22 to 37 in which R⁵ is selected from H, C₁₋₄-alkoxy and -CN, preferably MeO-.

20 39. A compound according to any of claims 22 to 37 in which R⁶ is H.

40. A compound according to claim 22 selected from:

3-(*tert*-Butyloxycarbonyl)-1-(chloromethyl)-1,2-dihydro-3*H*-benz[e]indole;

25 1-(chloromethyl)-3-[(5-methoxyindol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[e]indole;

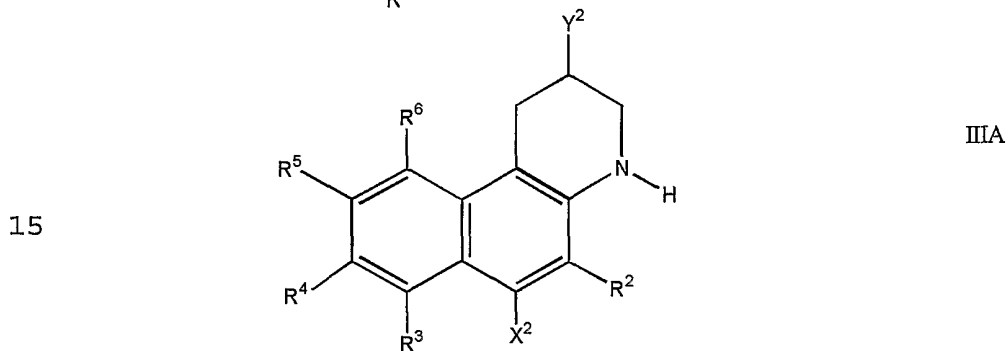
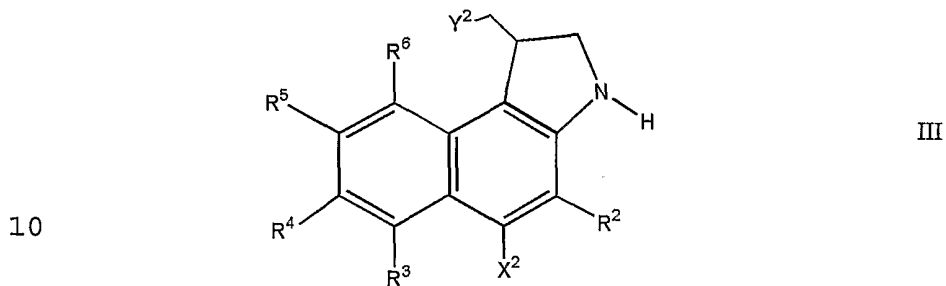
4-chloro-1-((*tert*-butyloxy)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline; and

4-chloro-1-((5-methoxyindol-2-yl)carbonyl)-1,2,3,4-tetrahydro-5,6-benzoquinoline.

30 41. A compound according to any of claims 22 to 40 for use in the treatment of an animal by therapy.

42. A pharmaceutical composition comprising a compound according to any claims 22 to 40 and a pharmaceutically acceptable excipient.

43. A synthetic method in which a compound of the formula III or IIIA

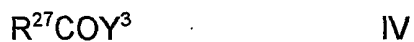


in which R², R³, R⁴, R⁵ and R⁶ are as defined in claim 1;

20 X² is H; and

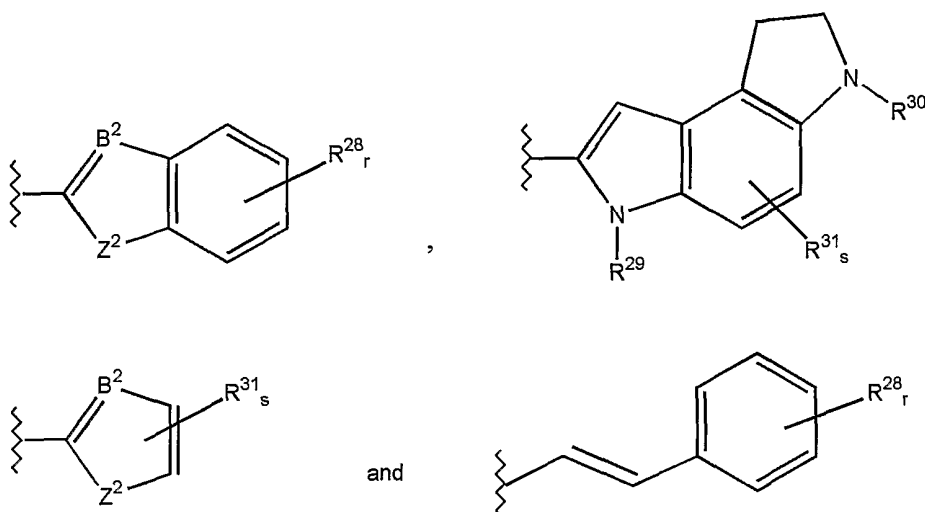
Y² is a leaving group or a hydroxyl or protected hydroxyl group;

is reacted with a compound of the general formula V



25 in which R²⁷ is selected from C₁₋₄-alkyl, optionally substituted phenyl, C₇₋₁₂-aralkyl, optionally substituted heteroaryl and Ar⁴;

Ar⁴ is selected from



in which B^2 is N or CR^{32} ;

Z^2 is O, S, $-CH=CH-$ or NR^{33} ;

the or each R^{28} is selected from C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, $-NHR^{33}$, $-NHR^{35}$, $-NR^{35}_2$, $-N^+R^{35}_3$, $-NHCOR^{34}$, $-COOH$, $-CONHR^{36}$ and $-COOR^{36}$;

r is an integer in the range 0 to 4;

R^{29} is an amine protecting group;

R^{30} is an amine protecting group, $-CONH_2$, $-COOH$, $-COR^{36}$ or $-COAr^5$;

the or each R^{31} is selected from C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, $-NHR^{33}$, $-NHR^{35}$, $-NR^{35}_2$, $-N^+R^{35}_3$, I, $-NHCOR^{34}$, $-COOH$, $-CONHR^{36}$ and $-COOR^{35}$;

s is 0, 1 or 2;

R^{32} is, selected from H C_{1-4} -alkoxy, C_{1-4} -alkyl, NO_2 , CN, Cl, Br, I, $-NHR^{33}$, $-NHR^{35}$, $-NR^{35}_2$, $-N^+R^{35}_3$, $-NHCOR^{34}$, $-COOH$, $-CONHR^{36}$ and $-COOR^{36}$;

R^{33} is an amine protecting group;

R^{34} is selected from Ar^5 , C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl optionally substituted heteroaryl and a ligand;

R^{35} is selected from C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl;

R^{36} is selected from C_{1-4} -alkyl, optionally substituted phenyl, C_{7-12} -aralkyl, optionally substituted heteroaryl and a ligand;

Ar^5 is selected from the same groups as Ar^4 , and

Y^3 is a leaving group;

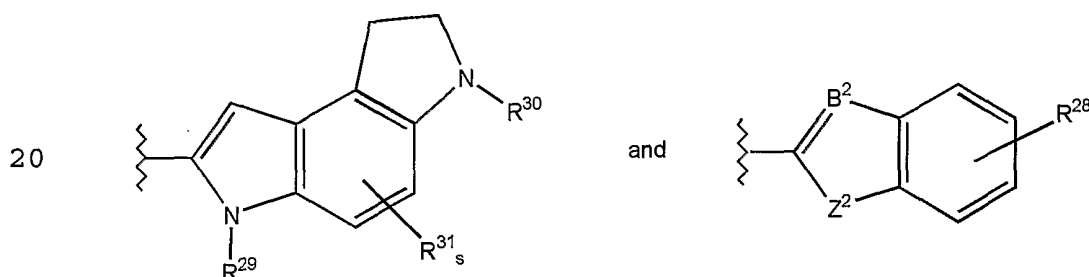
5 provided that no more than one R^{28} or R^{31} is any one ring is $NHCOAr^5$.

44. A method according to claim 43 which is carried out in the presence of an amide coupling reagent.

45. A method according to claim 43 or 44 in which the product is subsequently subjected to an amine deprotection step in which any or all groups R^{29} (if any) and/or any or all groups R^{33} (if any) are replaced by H.

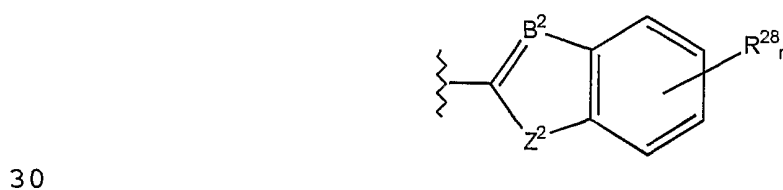
46. A method according to any of claims 43 to 45 in which Y^2 is selected from $-OCOOR^{17}$, $-OCONHR^{18}$, Cl, Br, I, and $-OSOOR^{19}$, in which R^{17} , R^{18} and R^{19} are selected from C_{1-4} alkyl, optionally substituted phenyl, C_{7-12} -aralkyl and optionally substituted heteroaryl; preferably Cl.

15 47. A method according to any of claims 43 to 46 in which Ar^5 is selected from



48. A method according to any of claims 43 to 47 in which R^{27} is Ar^4 .

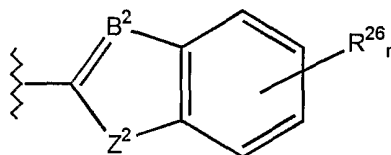
25 49. A method according to claim 48 in which Ar^4 is a group



50. A method according to claim 49 in which, in R^{27} , r is at least 1 and one of the groups R^{28} is $-NHCOAr^5$.

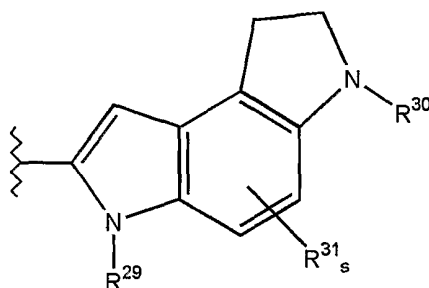
51. A method according to claim 50 in which Ar^5 is

5



52. A method according to claim 48 in which Ar^4 is a group

10

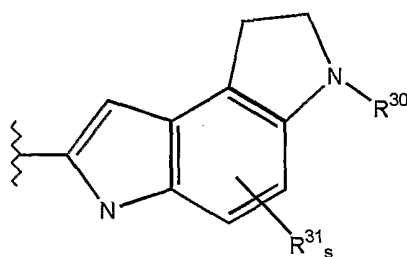


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53. A method according to claim 52 in which R^{30} is $-COAr^5$.

54. A method according to claim 53 in which, in R^{27} , Ar^5 is a group

20



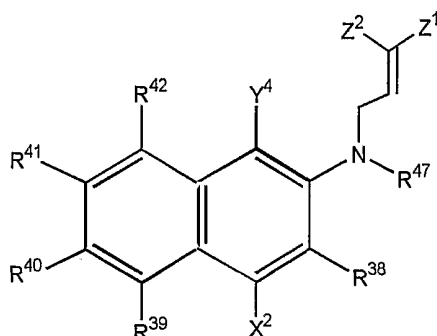
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55. A method according to any of claims 43 to 54 in which the compound of the formula III or IIIA is produced in a series of preliminary steps comprising a cyclisation step in which a compound of the general formula VI

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40

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VI

10 in which R³⁸ through R⁴², X² and Y² are the same as in the compound of the formula III;

R⁴⁷ is an amine protecting group,

one of Z¹ and Z² is Y⁵ and the other is H;

Y⁵ is a leaving group which is different from or the same as Y² and

Y⁴ is a radical leaving group;

15 is cyclised via an arylradical-alkene cyclisation step in the presence of a catalyst.

56. A method according to claim 55 in which Z¹ is Y² and in which the cyclisation step is carried out in the presence of a free radical to form a dihydropyrole ring.

20 57. A method according to claim 55 in which the free radical is generated from azoisobutyronitrile or is a 2,2,6,6-tetramethylpiperidinyloxy free radical.

58. A method according to claim 56 or 57 in which the catalyst is tributyl tin hydride.

25 59. A method according to claim 55 in which Z² is Y⁵, Y⁵ is a trialkyl tin radical, and the cyclisation step is carried out in the presence of a palladium complex to form a tetra hydroquinoline, which is oxidised to form an epoxide, the epoxides then being reduced to form an alcohol compound if Y² is other than hydroxyl, the hydroxyl group is subsequently converted into

30 Y².

60. A method according to any of claims 55 to 59 in which Y⁴ is a halogen, preferably I or Br.

INTERNATIONAL SEARCH REPORT

 International Application No
 PCT/GB 02/00801

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K31/40 C07D209/90 C07D221/10 A61K31/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 32850 A (SCRIPPS RESEARCH INST ;BOGER DALE L (US)) 12 September 1997 (1997-09-12) page 8, line 1 -page 9, line 4; examples 7-10 ---	1-60
Y	WO 97 12862 A (SCRIPPS RESEARCH INST ;BOGER DALE L (US)) 10 April 1997 (1997-04-10) page 33, line 1-27 page 36, line 17-29 ---	1-60
Y	WO 98 11101 A (DENNY WILLIAM ALEXANDER ;TERCEL MOANA (NZ); ATWELL GRAHAM JOHN (NZ) 19 March 1998 (1998-03-19) page 5, line 17-19 ---	1-60
	-/--	

 Further documents are listed in the continuation of box C.

 Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

7 June 2002

Date of mailing of the international search report

17/06/2002

Name and mailing address of the ISA

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Authorized officer

Engl, B

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 44000 A (PANORAMA RES INC) 27 November 1997 (1997-11-27) page 3, line 1 ---	1-60
Y	ATWELL G J ET AL: "Synthesis and cytotoxicity of amino analogues of the potent DNA alkylating agent seco-CBI-TMI" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 7, no. 12, 17 June 1997 (1997-06-17), pages 1493-1496, XP004136243 ISSN: 0960-894X the whole document ---	1-60
Y	BOGER D L ET AL: "DESIGN, SYNTHESIS, AND EVALUATION OF CC-1065 AND DUOCARMYCIN ANALOGS INCORPORATING THE 2,3,10,10A-TETRAHYDRO-1H-CYCLOPROPAUD BENZOUFQUINOL-5-ONE (CBQ) ALKYLATION SUBUNIT: IDENTIFICATION AND STRUCTURAL ORIGIN OF SUBTLE STEREOELECTRONIC FEATURES THAT GOVERN REACTIVITY AND REGIOSELECTIVITY" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US, vol. 116, no. 25, 1994, pages 11335-11348, XP002914133 ISSN: 0002-7863 page 11337, left-hand column, line 1 ---	1-60
Y	BOGER D L ET AL: "A POTENT, SIMPLE DERIVATIVE OF AN ANALOG OF THE CC-1065 ALKYLATION SUBUNIT" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 1, no. 1, 1991, pages 55-58, XP000655071 ISSN: 0960-894X the whole document ---	1-60
Y	BOGER D L ET AL: "CC-1065 CBI analogs: an example of enhancement of DNA alkylation efficiency through introduction of stabilizing electrostatic interactions" BIOORGANIC & MEDICINAL CHEMISTRY, ELSEVIER SCIENCE LTD, GB, vol. 3, no. 6, 1995, pages 611-621, XP002086064 ISSN: 0968-0896 the whole document ---	1-60

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INTERNATIONAL SEARCH REPORT

 tional Application No
 PCT/GB 02/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BOGER D L ET AL: "DUOCARMYCIN-PYRINDAMYCIN DNA ALKYLATION PROPERTIES AND IDENTIFICATION, SYNTHESIS, AND EVALUATION OF AGENTS INCORPORATING THE PHARMACOPHORE OF THE DUOCARMYCIN-PYRINDAMYCIN ALKYLATION SUBUNIT. IDENTIFICATION OF THE CC-1065-DUOCARMYCIN COMMON PHARMACOPHORE" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US, vol. 112, 1990, pages 8961-8970, XP002058066 ISSN: 0002-7863 the whole document ----	1-60
Y	BOGER D L ET AL: "SYNTHESIS AND EVALUATION OF CC-1065 AND DUOCARMYCIN ANALOGUES INCORPORATING THE ISO-CI AND ISO-CBI ALKYLATION SUBUNITS: IMPACT OF RELOCATION OF THE C-4 CARBONYL" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 62, no. 25, 1997, pages 8875-8891, XP000915623 ISSN: 0022-3263 the whole document ----	1-60
A	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; retrieved from STN Database accession no. 89:146737 XP002187817 abstract & KUTKEVICIUS, S. ET AL.: "Study of the acylation of 1,2,3,4-tetrahydroquinoline hydroxy derivatives" DEPOSITED DOC. VINITI, 1976, pages 1108-1176, ---- -/--	43-60

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; retrieved from STN Database accession no. 88:169883 XP002187816 abstract & STEPANIUKAS, A.; SERENAS, K.: "Synthesis and some reactions of benzo'e!- and 5-methylbenzo'g!-derivatives of 1a,2-dihydro-1H-azirino'1,2-a!indole" CHEM. CHEM. TECHNOL., TECH. MOKSLU ISVYSTYMO RESP. JU REZULT. PANAUDOJIMO KONF. MEDZIAGA, 1975, pages 99-100, -----</p>	43-60

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/00801

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9732850	A	12-09-1997	AU 711974 B2	28-10-1999
			AU 1990297 A	22-09-1997
			CA 2246783 A1	12-09-1997
			EP 0888301 A1	07-01-1999
			JP 2000506168 T	23-05-2000
			WO 9732850 A1	12-09-1997
			US 5985908 A	16-11-1999
WO 9712862	A	10-04-1997	AU 727608 B2	14-12-2000
			AU 7431596 A	28-04-1997
			CA 2233936 A1	10-04-1997
			EP 0862553 A1	09-09-1998
			JP 11513391 T	16-11-1999
			NZ 321172 A	28-02-2000
			WO 9712862 A1	10-04-1997
WO 9811101	A	19-03-1998	AU 721037 B2	22-06-2000
			AU 4403997 A	02-04-1998
			EP 0938474 A2	01-09-1999
			JP 2000517292 T	26-12-2000
			WO 9811101 A2	19-03-1998
			NZ 334344 A	25-08-2000
WO 9744000	A	27-11-1997	US 5843937 A	01-12-1998
			AU 3217097 A	09-12-1997
			CN 1219841 A	16-06-1999
			EP 0918752 A2	02-06-1999
			JP 2000511893 T	12-09-2000
			WO 9744000 A2	27-11-1997